"Effect of protein and carbohydrates ratio on polymorphic inversions in *Drosophila ananassae*"

ड्रोसोफिला एनानेसी मे बहुरूपी व्यूत्क्रम पर प्रोटीन और काबोहाइड्रेट के अनुपात का प्रभाव

SHORT SYNOPSIS

Submitted in partial fulfillment of the requirement of the degree of

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To

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By

Radha Krishna Gupta

En. No. 2301702

Under the Supervision of

Prof. J. P. Yadav

School of Sciences

Subject – Zoology

School of Sciences

U. P. Rajarshi Tandon Open University

Shantipuram, (Sector – F) Phaphamau, Prayagraj - 211021

School of Science

Certificate

I hereby declare that the short synopsis **"Effect of protein and carbohydrates ratio on polymorphic inversions in *Drosophila ananassae*"** being submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Zoology under school of sciences of **U.P. Rajarshi tandon Open University, Prayagraj** during the academic year **2024-25**

It is a bonafide record of original work carried out under the supervision of Prof. J. P. Yadav and has not been submitted in full or part to any other university of institute for a degree or diploma. Further, it is certified that the short synopsis is plagiarism-free and the report has been submitted.

Signature

(Radha Krishna Gupta)

(En. No. – 2301702)

Research Supervisor Signature 6/5/25

Prof. J. P. Yadav

Faculty of Science

(Signature) 06/05/25

Name - Dr. Ashutosh Gupta

(Director of Concerned School of Studies)

(Signature 06/05/25

Name – (Prof. P. K. Pandey)

(Secretary of RDC/Director, R&D Cell)

Abstract

Drosophila, a fruit fly is a well-known model organism. Inversions are wide spread and they do not harbor deleterious mutation and maintained as polymorphism by directional selection. Inversion plays key role in local adaptation because of their effect in recombination. Protein and carbohydrates diet ratio may work as local environment factor and thus could be responsible for change in frequencies of polymorphic inversions in *Drosophila* and therefore a new adaptive value may be added in fly's survival. Protein and carbohydrates ratio (P:C) may also affect the development of particular phenotype response depend upon specific ratio of P:C. Thus P:C ratio should also be responsible in shaping and influencing fitness trait like fecundity, body weight, walking performance assay, longevity, desiccation resistance in *D. ananassae* effected by polymorphic inversions frequencies. This work proposes to study different P:C ratios in context to polymorphic inversions and changes occurred in fitness traits in *D. ananassae* to understand the adaptive significance for better survival as a whole.

Key words- Drosophila, Polymorphic inversions, Fitness trait, Trade-off, Adaptation.

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(1) Introduction

Drosophila, a fruit fly is a well-known model organism. It is economical and is very effective for studies ranging from behavior to molecules and evolution, cell biology to development biology, human disorder and even social activities that too, at molecular level. It was once considered as the best model of genetics as many studies on genetics were investigated on *Drosophila.* For the common people, *Drosophila* means Drosophila melanogaster, but there are 1500 species reported in this genus (Singh 2013). Among all species of *Drosophila, D. funberis* was first described in 1787 by J.C. Fabricus and was moved in to the genus *Drosophila* by C.F.Fallen in 1823. Similarly, *melanogaster* was described by Mayer in1830. By the early 1900, *D. melanogaster* became an established model organism, but researcher realized the importance of several other species of *Drosophila* also. In the late 1930Th.Dobzhansky begin to use D. pseudoobscura and its sibling species *D.persimiles* and *D .miranda* for the classical evolutionary and population genetics studies (Anderson et al, 1991).*D. subobscura* is in many ways the old word counterpart of the*D. Pseudoobscura* (Powell 1997) and it has been extensively studied for inversion polymorphism by European researchers such as Prevosti, Sperlich.Among all these species. *ananassae*has also attracted the attention of a number of researcher particularly from Japan, USA, France, Germany and India .*D. ananassae* is cosmopolitan and domestic species, and occupies a unique status among the *Drosophila* species due to certain peculiarities in its cytological and genetic behavior, spontaneous male combination, distortion varied chromosomal polymorphism and occurrence of spontaneous genetic mosaic (Singh, 2013). Singh and Mohanty (1992) detected a spontaneous balanced genetic mosaic in *D. ananassae* having three mutant characters. It was a male fly and mitotic recombination in the zygote considered as the origin for this mosaic.

D. ananassae belongs to the ananassae species Complex of the ananassae subgroup of the melanogaster species group. It uniqueness has also provided by a number of studies as it occupies a separate branch in *Drosophila* phylogeny. The chromosomal polymorphism has studies extensively in the laboratory population of *D ananassae.D. ananassae* was initially described by Doleschall in 1858 from an Iceland in Indonesia. It was cosmopolitan in distribution and is largely circumtropical and of frequent occurrence in domestic habitat. It is recorded from all six biogeographic zone, but it is absent in some areas (Singh 2010). It is also present in India .*D. ananassae* is present in different localities and place in India. It is used in food and nutrition researches as 75 percentage of the fruit fly gene are orthologous to

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mammals. Metabolic and signal transduction pathway is highly conserved in *Drosophila.* It feeds upon yeast, corn and sugar. It is also called holistic diets (Laverson and Stata et.al., 2018). Some researchers study on chromosomal polymorphism due to paracentric inversion in natural population of *D. ananasae.* They suggested that enzyme polymorphism has been used mainly to detect selection acting on specific loci, to understand genetic structure of population and to analyze the pattern of Geographical distribution. They observed that number of copies and alloenzyme variation are higher in *D ananassae* (De Lage et.al 1992.) An analysis sub group including *D ananassae.* Itself has shown that a maximum of 30% are polymorphic and that even most polymorphic (Estc,AcPh,Ca,Pgm) loci show similar variability (Tobari 1993).

Inversions are portion of the chromosomes whose genes order has been reversed relative to the standard orientation. Crossing over is strongly suppressed between two pair chromosome that differ by an inversion. Heterozygosity for an inversion induces a loop structure in pair impaired chromosome and thus inversions are easy to detect cytological. This convenient feature motivated the original studies in *Drosophila.* Inversion have known function. they were first considered to be subject primarily to genetic drifts however, selective effects associated with inversion were soon discovered. The first evidence of a chromosome inversion was found in 1921, in this year *D. melanogaster* inversion were regarded as recombination suppresser. Inversion takes place in polytene chromosomes in *D melanogaster* third instar larvae salivary gland. Th. Dobzhansky proposed that inversion has higher fitness than those that are found outside of the inversion. Some observation indicates that diets affect the *Drosophila* reproduction and survival rate. This also indicates that proteins affect the reproduction rate of female Drosophila and take a large amount of protein (Mathew and Corey et.aL).

(2) Literature Review -Nutrigenomic study is based upon nutritive material and its introduction on the genome. It influences of nutrients on F genes expression is called nutrigenomic. It represents of gene variant to nutrients, dietary component and developing nutraceuticals called nutrigenices. It has been demonstrated that numerous genetic polymorphism can influence protein structure. Nutrients act as environmental factors and can interact to genetic material. Nutrient and nutrient component help the DNA replication and

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repair mechanism, dietary material act as cofactor or substrate in metabolic pathway (Farad and Zarf, 2010).

Genetic polymorphism influences to environmental condition such as temperature, protein components, lipids concentration etc. Nutrients can affect the development of a particular phenotype. Thus, response depend on specific nutrient that determined by the individual genotype. The Control rate of genetic code in determining genome stability and health related outcome such as degenerative disease and causes are well understand by amino acid and its related proteins due to effect of polymorphic inversion. These specifically the foetal basis of adult nutrition and other environmental factor during parental and post parental development influenced by gene expression and alter their function which can change into harmful disease.

These gene expressions influenced the chromosomal stability, metabolic pathway and their product, signal transduction and their related pathway, protein synthesis event and many more epigenetic events through protein and their derivative product like enzyme, hormone and other by product.

Chromosomal inversion in which a linear DNA sequence is flipped in its orientation are a common member of the menagerie of DNA polymorphism and have found in diverse organism's population such as human plants and fruit fly (Krimbas and Powell et.al.1972)

Inversions are wide speeds and they do not harbor deleterious mutational leads and maintained as polymorphism by directional selection. Deer mice revealed 13 inversions between population, of which have exhibit significant traits implicated in local population. Inversion play key role in local adaptation because of their effect in recombination.

My work is based upon effect of protein and carbohydrates ratio on polymorphic inversion in *Drosophila ananassae*. Protein and carbohydrates diet ratio is working as local environmental factor just like low pH, high pH, higher temperature, lower temperature regional climatic condition drought and other ecological factor. these factor are responsible for polymorphic inversions in *D ananassae*. This inversion makes protein like product enzyme, cofactor, different metabolic pathway and signal transduction pathway change. Which can change related product and change in the body activity of *Drosophila. These* changes result may disease like cancer, Diabetes many syndromes related in the human being.

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(3) Description Broad Area-

(1) Stribytska, Valiank (2020),work on parental dietary protein to carbohydrates ratio affects offspring's life span and metabolisms in *Drosophila*. In their experiments they feed a group with different ratio of protein carbohydrates. They observe that parental protein to carbohydrates ratio affected the life span of female offspring. An increase in the protein carbohydrate ratio from 0.3 to 0.65 decreased the level of glucose trehalose in the offspring's and a similar tendency was observed in level of circulating hemolymph glucose and trehalose. Offspring also accumulated lees triglycereldehyde but move glycogen when parent were feed a low protein carbohydrates diet. This study indicates that the parental diets protein carbohydrates ratio has strong impact on the life span reproduction appetite and metabolism the offspring generation.

(2) Farhud, et.al (2019) reported that neutrional genomics focused on interact between bioactive food components and neutrogenomices.

(3) Eriction and Halaka (1976) demonstrated that polymorphisms at 25 loci coding for liver enzyme was studied in two feral three outbreed and three inbreed rats strain by starch gel electrophoresis.

(3.1) Significance of study-The study of the effect protein and carbohydrates ratio on polymorphic inversion in *Drosophila* is significant for several regions:

1-Understanding genetic variation and adaptation: polymorphic inversions are structural variations in chromosomes that can influence an organism's fitness and adaptability. By studying how dietary factors like protein and carbohydrates ratio affects these inversions, researcher can gain insights in to the mechanism of genetic variations and how environmental factors drive evolutionary change.

2-Nutrional Genomics: This research contributes to the field of nutrional genomics., which explores how diet interacts with genes to influence health and development. Understanding the impact of macronutrient balance on genetic structures like polymorphic inversion.

3-Metabolic pathways and physiology: Different ratio of proteins and carbohydrates can significantly alter metabolic pathways and physiological states in *Drosophila*. Investigating these effects can reveal how nutrients availability influences genetic and phenotypic plasticity, shedding light on the metabolic demands and adaptability of organisms.

4-Evolutionary Biology: The study provides valuable information on how dietary factors can drive natural selection and evolution. By examining how different protein and

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carbohydrates ratio affect genetic structure researcher can better understand the evolutionary pressure that shape population over times.

(3.2) Research questions:

1-What is the effect of protein and carbohydrates different ratio on *Drosophila ananassae* on polymorphic inversions?

2- What is the effect of protein and carbohydrates different ratio on *Drosophila ananassae* on related physiological and biochemical process?

3- What is the effect of protein and carbohydrates different ratio on *Drosophila ananassae* in their related traits like fecundity, desiccation resistance, body size Walking capacity etc.?

4- What is the effect of protein and carbohydrates different ratio effects on *Drosophila ananassae* in the sense of trade-off?

(3.3) Statements-Effects of protein and carbohydrates ratio on polymorphic Inversions in *Drosophila ananassae.*

(3.4)-Operational definitions and key terms-

3.4-a-Polymorphic inversions: Polymorphic inversions is chromosomes arrangements in which a segments of a chromosomes inverted with in original position. chromosomes break two positions within chromosomes. These inversions are two types-

a-Para centric inversions: In this inversions not include centromere and both break point occurs in one arm of the chromosomes.

b-Pericentric inversions: In this inversions centromere includes and therefore, break in each arms.

3.4-b -Carbohydrates-Optically active polyhydroxy aldehyde or ketones or the Compound which produce of such type hydrolysis.

3.5-c-Protiens-Protiens are cellular macromolecules made up of amino acids. We used different amount of proteins and carbohydrates ratio.

3.5-d -Fecundity- Total number of eggs laid in a definite period of bouts.

3.5-e - Desiccation resistance- Desiccation resistance is the ability of an organism to reduce water Loss or withstand extreme dryness.

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3.5-f - Trade-off: A trade –off exists when one trait cannot increase without a decrease in another (or vice Versa). Such a situation can be caused by a number of physical and biological

mechanisms.

3.6-g - Longevity: Length/period of life from birth (eclosion in this case) to death of the organism.

(4) Objectives of study:

1- To study protein and carbohydrates ratios impact on polymorphic inversions in *D. ananassae.*
2- To study the effect of protein and carbohydrates ratios on biochemical traits and trade-offs among the in *D. ananassae.*
3- To study fitness traits like fecundity, body size, walking capacity and desiccation resistance in *D. ananassae* due to effect of protein- carbohydrate ratios.

4- To study adaptive effects due to polymorphic inversions on different fitness traits in context to protein- carbohydrate ratios in *D. ananassae.*

(5) Research Methodology:

Research method-

***Drosophila* stocks-** In this study, *D. ananassae* flies from a natural population (with at least 25 females), one laboratorybase population (made from latitude wise 5 different populations) and two homozygous lines (ST/ST as standard and AL/AL as recessive) for Alpha inversion (available in the laboratory) will be used. For culturing, the flies will be kept at room temperature. If room temperature will not be adequate, the cultures will be kept in a BOD Incubator.

Preparing *Drosophila* food media-The commonly used food media for *Drosophila* stock maintenance include Agar-agar, Yeast extract, Maize flour, crude sugar, Nepagine (Methyl-p-hydroxy Benzoate) and Propionic acid.

To prepare fly media, Agar-agar is added to the hot water to make a clear gel. Following this, Yeast extract, Maize flour, sugar, and Nepagin are added. After 10-15 minutes of cooking, heat should be turned off. Once the food media temperature reaches to around 50-degree centigrade Propionic acid is added and mixed properly. Throughout the preparation of food constant stirring is required. Yeast hold special nutritional value for *Drosophila*, while

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Nepagine is the antifungal in nature and Propionic acid is a bactericides and functions as preservative and increases the self-life of the food. The food could then immediately be transferred to the sterilized vials or bottles or petri dish as soon as the media starts solidifying and then stored in at a cool place. Any moisture in bottle or vial should be removed before use.

Detail of food media: Drosophila food media

Items	1 unit
Agar-agar	36 g
Maize powder	108 g
Crude Sugar	96 g
Yeast powder	36 g
Nepagin (Methyl-p-hydroxy Benzoate)	8 g
Propionic acid	8 ml
Water	2400 ml

Identification-For adult male and female identification of *D. ananassae* see the Atlas map of this fly. Tobari (1992). Flies are too small in size to be observed with naked eyes. Hence magnification is required and flies are observed under Stereo zoom microscope. Flies are anaesthetized with the help of ether.

***Drosophila* egg collection** –Allow *Drosophila* to lay eggs overnight on to a petri dish containing agar supplement. To remove the eggs, add distilled water to dish and gently wipe the surface with a soft paint brush to collect the eggs.

Protein-Carbohydrate (P:C) ratios:

Seven (6 experimental- 200, 150, 125, 75, 50 & 25% and one normal/ control-100%) P: C ratio diets with 10 flies per group having 5 males and 5 females in each vial (of 25mm diameter and 100mm length, with 6ml food), will be conducted for each set of experimental groups (Natural, laboratory base populations, Alpha-ST/ST, and Alpha-AL/AL (each in triplicate). Virgin flies will be collected over a 48-h period and after 3 days allowed to mate for 24 h and then transferred to experimental diet. All these groups will be transferred to next vial containing experimental diet for 3 times, in order to get a

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sufficient number of eggs for larvae and to get flies for next generation as well as for biochemical analyses. For the one unit of food (2400 ml), 108g maize powder and 96g sugar are used as the source of carbohydrate, while 36g yeast extract powder is used as the source of protein. The water content will be fixed 2400 ml, therefore at the higher ratios, there shall be a limitation of water in food, and this will suggest the role of water also compounded with the diet. Agar-agar (36 g), nipagin (8g) and propionic acid (8ml) will also be used in the experimental diet; the latter two items are used as a fungal suppressor. Due to limitations of data of autolyzed yeast powder and sugar used in the study, calorie content provided by each treatment diet will not be calculated as Lee et al., 2008. These flies will be maintained at 25^0C with minimum 65% humidity in a BOD incubator.

Polytene Chromosome and Inversion study:

Chromosomal analyses of third instar larvae will be done by taking 25 larvae randomly from each culture bottle of each diet set, using Lacto-Aceto-Orcein method under microscope and data will be recorded by a microscopic camera. Larvae will be dissected under Stereo zoom binocular. While studying the chromosome inversion polymorphism, 25 flies will be taken randomly from each diet culture and these will be maintained separately for two generations before the study, on an acid mix medium containing agar-agar, active yeast, maize powder, crude sugar, nipagin, acid mix and water. The acid mix contains propionic acid, phosphoric acid and water. It is used to get healthy larvae and thus gives very good polytene chromosome preparations. Data will be recorded for all three polymorphic inversions present and frequency (in natural, base population and homozygous lines) will be calculated to know the fluctuations if any.

Fecundity test:

For fecundity test, 3 days old 50 virgin flies (25 males and 25 females) in two sets of each will be allowed to mate for 48 hours. The fecundity will be recorded as the total number of eggs laid by 25 females of each set in 12 bouts, each of 24 hours' duration with a gap of 3- 4 h between two bouts. Eggs laid during the gap periods were also included in the total fecundity counting.

Desiccation test:

For desiccation tests, eclosed virgin flies will be collected within 2- 6 hours of eclosion and will be taken in 2 sets, each with 10 males and 10 females from each diet plan. Flies will be put in empty vials with a desiccant during the test period and will be monitored at every two hours until all flies are dead.

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Body size

Body size is used as a proxy for correlated responses. For body size measurement, just after the fecundity test, 25 males and 25 female flies will be picked up randomly. Body size will be measured in the form of thorax length with the help of the ocular micrometer in units (1 Unit =16. 67μ). The thorax length will be measured from the anterior part of the thorax (neck) to the distal tip of the scutellum of flies of each experimental diet.

Walking performance assay:

For walking performance assay, 10 (x3) virgin flies will be placed into vertically oriented plastic tubes (25mm internal diameter and 200mm long). After a 10min recovery period, the flies will be shaken down to the bottom of the tube. After 10 secs, a number of flies able to climb near the top of the column over 15cm (ntop) and a number of flies remaining at the bottom or near the bottom from 0-15 cm (nbot) of the column will be recorded. The test will be repeated five times for each set at 1min intervals. Mean walking performance index will be calculated for each diet treatment of each category of flies, using the formula [1/2[(ntot+ntop-nbot)/ntot].

Lipid content:

For lipid content analyses, from each diet, 10 males and 10 females in two sets will be taken, kill by ether; measure wet body weight, dry for 36 hours at 70^0C, and then weigh again (called dry weight) prior to lipid extraction. After weighing, flies of each batch of each diet will be kept in 6 ml of diethyl ether with gentle agitation for 48 hrs. on rocker shaker. The ether is then removed, and the flies will again be dried for 12 hrs. at 70^0C and weigh (called lean weight). The difference between dry weight and lean weight of flies of each batch will be taken as the weight of lipid content.

Carbohydrate content:

For carbohydrate content analyses of each diet group, flies will be taken in two sets, each with 10 flies per sex. Flies will be killed by over etherization and wet weight will be measured by a microbalance, dry for 36 hours at 70^0C, and weigh again as dry weight prior to carbohydrate estimation. Flies of each batch will then be crushed in small volumes of distilled water, and the final volume will be made up to 1 ml. These samples will then be incubated in a water bath at boiling temperature for 30 minutes. After filtration, 50 μl of the filtrate in each sample will be taken and 3 ml of freshly prepared Anthrone solution will be added to it. Samples will then be heated in a water bath at boiling temperature for 30 minutes and allowed to cool at room temperature. The optical density will be measured through a spectrophotometer at 630 nm wavelength. The total

glucose concentration will be estimated from a glucose standard graph constructed for the assay.

Protein content:

For total protein content analyses of each diet group, flies will be taken in two sets, each with 10 flies per sex. BCA method will be applied and the optical density will be measured at 562nm.

Longevity: For this study, 100 virgin flies of each sex will be collected from each diet group and will be kept with the same group diet at a density of 25 flies/ vial. They will be transferred to new respective food vials every 5th day. Dead flies will be counted every day but will be removed during subsequent transfer of flies to new food vial. This procedure will be repeated until all flies will be dead. Data will be recorded as the numbers of flies remain alive at each counting.

Procedure of Data Collection and statistical method:

Differences in mean, fecundity, protein, carbohydrates, desiccation resistance body size, and longevity among different populations will be tested by One-way ANOVA and interaction among different traits will be analyzed by Two-way ANOVA (Zar,2003). Chromosomes inversion frequencies will be calculated from the inversion karyotype and differences with different attributes will be calculated because inversion karyotypes are suggested as normally distributed. (Herman and Parson, 1981). Trade-off will be studied among different traits by Pearson correlation and their functional analysis will be done by studying regression analysis (Zar,2003).

(6) Expected Out Come

Understanding the influence of protein and carbohydrates ratio on polymorphic inversion dynamics in *Drosophila* provides insights in to the mechanisms driving genetic variations and adaptation in natural population. This knowledge may have broader implications for evolutionary biology and human health, as similar mechanisms could operate in other organism, including humans, influencing susceptibility to genetic diseases and responses to dietary interventions for their better survival.

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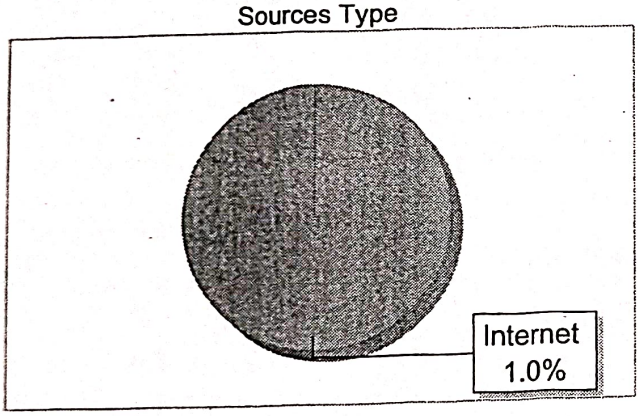

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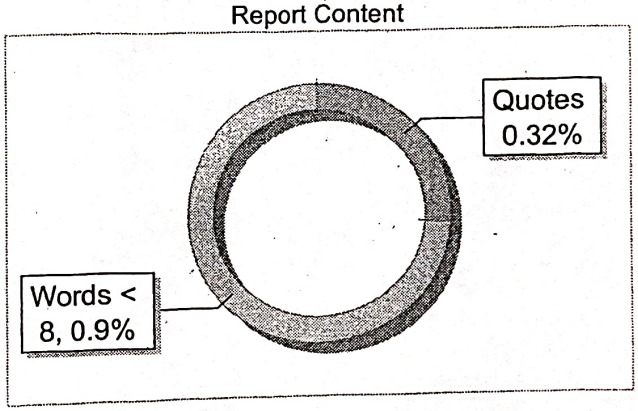

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